Gene Regulation by Histone H1: New Links to DNA Methylation

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Linker histones of the H1 family are among the most abundant components of chromatin. In this issue of *Cell*, Fan et al. (2005) examine the effect of H1 downregulation on gene expression. Although a 50% reduction of histone H1 levels in embryonic stem cells affects chromatin structure globally, the expression of very few genes is altered. Intriguingly, this study reveals a new link between H1 and DNA methylation.

H1 linker histones anchor themselves to nucleosomes with their central, globular domains, while their highly positively charged C-terminal domains reach out to organize the linker DNA on either side of the nucleosome. This interaction promotes the folding of nucleosomal arrays into 30 nm fibers in vitro (Jerzmanowski, 2004). Simultaneously, association of H1 with nucleosomes prevents the unpeeling of DNA from the histone surface at the point of entry of the DNA into the nucleosome. This association hinders both spontaneous dynamic transitions of nucleosome structure and active, ATP-dependent nucleosome remodeling. The suppression of nucleosome dynamics and the compaction of the nucleosomal array during folding by H1 suggest that this linker histone may act as a global repressor of transcription. However, the available biochemical data on this issue are controversial (Sandaltzopoulos et al., 1994 and references therein).

In many cells, the cumulative abundance of all H1 variants suggests that each nucleosome is bound by one linker histone. Therefore, H1 was long thought to be an obligatory, structural component of chromatin. However, on closer inspection, both the linker-to-core histone stoichiometry and the relative abundance of the individual H1 isoforms vary significantly between cell types (reviewed by Zlatanova et al., 2000). Most strikingly, Fan et al. (2003) determined that mouse embryonic stem (ES) cells contain only one linker histone for every two nucleosomes. Considering the highly dynamic interaction of H1 with nucleosomes (Jerzmanowski, 2004), we assume that chromatin in ES cells is much more flexible than that of well-differentiated cells, which may correlate with its higher developmental potential. Interestingly, targeted inactivation of any single one, or even two, of the six somatic H1 genes present in mice (Zlatanova et al., 2000) fails to reduce the H1 complement in mouse ES cells due to a compensatory increase in other H1 isoforms. The simultaneous inactivation of three H1 genes (H1a, H1b, and H1c) in compound mutants is required to achieve a 2-fold reduction of total H1 abundance, and this comes at a high price. Such triple-H1 mutant embryos die by mid-gestation with a broad range of defects (Fan et al., 2003). Thus, maintenance of a minimal amount of linker histones is essential in mammals.

In this issue of *Cell*, Skoultchi and colleagues take the analysis one important step further by investigating the chromatin structure and gene-expression profile of ES cells derived from the triple-H1 mutant embryos (Fan et al., 2005). Similar to the parental embryos, these cell lines have a total H1-to-nucleosome stoichiometry of 0.25. Not unexpectedly, this low H1 abundance has some global consequences on chromatin architecture such as a pronounced shortening of the average nucleosome repeat length and reduced nucleosomal H4K12 acetylation levels. As the authors discuss, these effects may serve to counteract the change in charge balance in chromatin caused by the depletion of H1.

Surprisingly, however, these global chromatin changes are not accompanied by major alterations in the transcriptome of the H1 triple mutant cells. Carefully controlled microarray analysis shows that only about one in 200 genes is expressed differently in the H1-depleted ES cells. Such highly selective effects of H1 on gene regulation are not restricted to mammals. In early *Xenopus* embryos, preventing the timely accumulation of somatic H1 protein has been shown to result in the selective derepression of oocyte 5S RNA genes. Furthermore, the developmental time period, during which the muscle master regulatory gene *myoD* (and subsequent muscle differentiation) can be induced from pluripotent embryonic cells, is significantly extended in the H1-deficient embryos (Steinbach et al., 1997 and references therein). Mechanistically, repression of myoD transcription by H1 appears to involve a physical interaction between the homeodomain protein Msx1 and the linker histone variant H1b, which together bind to a key regulatory element of the *myoD* gene and establish repression (Lee et al., 2004).

Interestingly, Skoultchi and colleagues (Fan et al., 2005) also found

that reducing the amount of linker histones by half in ES cells not only induced but also repressed several genes. Although indirect effects cannot be excluded at this stage, a positive effect of the binding of linker histones on transcription has recently been observed (Koop et al., 2003). In this case, H1 has been suggested to improve the precise positioning of a strategic nucleosome, which is a crucial prerequisite for synergistic activation of transcription by several regulators. In this respect, it is also remarkable that the H19 gene, which Fan et al. (2005) report to be particularly affected by H1 removal, appears to be bound by substoichiometric amounts of H1 even in wild-type ES cells. Conceivably, those few H1 molecules that are observed by chromatin immunoprecipitation play an important regulatory rather than a structural role in the regulation of this gene.

As a complete surprise, however, comes the observation that about one third of the genes sensitive to H1 depletion are known to be regulated by DNA methylation (9/29 genes), thus being significantly overrepresented in this group. These include both parentally imprinted genes and several gene loci on sex chromosomes. Strikingly, the authors show that global DNA methylation patterns are normal in the triple-H1 null ES cells, including repetitive, heterochromatic regions that are highly methylated. Instead, they observe a quantitative reduction in the extent of DNA methylation at specific CpG dinucleotides within the imprinting control regions of the *H19-Igf2* and *Gtl2-Dlk1* gene loci. These results suggest that a certain threshold of H1 protein is necessary to either maintain or establish some gene-specific DNA methylation patterns. Notably, knockdown of total H1 in *Arabidopsis* by RNA interference also led to some stochastic alterations in DNA methylation patterns (Wierzbicki and Jerzmanowski, 2005), indicating that a functional link between H1 and DNA methylation may exist also in plants.

Taken at face value the data suggest that the presence of H1 at specific sites increases DNA methylation levels locally, which could reflect a particular pathway toward repression. Given that a direct preferential interaction of H1 with methylated nucleosomal DNA has not been consistently observed (reviewed in Zlatanova et al., 2000), more indirect scenarios must be considered. For example, a DNA methyltransferase could be targeted to a site by H1 via a heterochromatin protein 1 (HP1) bridge (Craig, 2005). The interaction of HP1 with the linker histone may be regulated by posttranslational modification of the latter, in light of recent in vitro observations (Daujat et al., 2005). If histone H1 contributes to establishing a specific repression pathway, principles must be in place to assure the selective placement of H1 in ES cells, where the low levels of the histone are sufficient to organize only half of the chromatin. Under those circumstances, interaction of H1 isoforms with sequence-specific DNA binding proteins, as has been observed for H1b and Msx1 (Lee et al., 2004), may be relevant. Other possible targeting mechanisms include DNA sequence preferences and specific core histone modifications.

The gene-specific response following the experimental reduction of the linker histone H1 is reminiscent of the effects observed when the nucleosome density in yeast cells was reduced by half through downregulation of the core histone H4 (Wyrick et al., 1999). Surprisingly, only about 10% of the yeast genes were affected by this depletion of nucleosomes; however, a clear correlation between derepression and a telomere-proximal location of the corresponding genes was apparent. Obviously, telomeric silencing depends most critically on the underlying chromatin infrastructure.

The gene-specific response to ablation of H1 points to a pathway in which repression involves DNA methylation and relies critically on histone H1. So far, many molecular interactions have been described that culminate in DNA methylation, but none of them based on histone H1 (Craig, 2005). It is possible that the gene-specific modulation of transcription by H1 reduction has a physiological correlate in an activation mechanism that involves the selective removal of H1 from target genes. Available mechanisms include H1 phosphorylation, its poly(ADP-ribosyl)ation, the action of H1 chaperones, or the reduced affinity of linker histones to highly acetylated nucleosomes (Koop et al., 2003; Zlatanova et al., 2000).

This study by Fan et al. (2005) provides a particularly nice example of how an abundant chromatin constituent can contribute to gene-specific regulation. However, the regulatory potential of linker histones will only be fully appreciated once the different properties of the individual H1 variants and the effect of their posttranslational modifications have been elucidated.

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